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Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA

S. Kavitha [a], S. Adish Kumar [a], K.N. Yogalakshmi [b], S. Kaliappan [c], J. Rajesh Banu [a,*]

[a] Department of Civil Engineering, Regional Centre of Anna University, Tirunelveli, India
[b] Centre for Environmental Science and Technology, Central University of Punjab, Bathinda, India
[c] Department of Civil Engineering, Ponjesly College of Engineering, Nagercoil, India

HIGHLIGHTS

- Removal of EPS with 0.2 g/g SS of EDTA enhanced the bacterial pretreatment.
- Kinetic parameters of deflocculated sludge reveal efficient sludge reduction.
- Solubilization in deflocculated sludge was 11% higher than the control.
- SS reduction in "E" reactor which contains pretreated sludge was 48.5%.

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ABSTRACT

In this study, the effect of Ethylene diamine tetra acetic acid (EDTA) on Extracellular polymeric substance (EPS) removal tailed with bacterial enzymatic pretreatment on aerobic digestion of activated sludge was studied. In order to enhance the accessibility of sludge to the enzyme secreting bacteria; the extracellular polymeric substances were removed using EDTA. EDTA efficiently removed the EPS with limited cell lysis and enhanced the sludge enzyme activity at its lower concentration of 0.2 g/g SS. The sludge was then subjected to bacterial pretreatment to enhance the aerobic digestion. In aerobic digestion the best results in terms of Suspended solids (SS) reduction (48.5%) and COD (Chemical oxygen demand) solubilization (47.3%) was obtained in experimental reactor than in control. These results imply that aerobic digestion can be enhanced efficiently through bacterial pretreatment of EPS removed sludge.

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1. Introduction

The Waste activated sludge (WAS) produced from the biological waste water treatment process has dramatically increased in recent decades due to quantitative and qualitative expansion of waste water treatment (Yang et al., 2010).The increasing amount of WAS derived from waste water treatment process had become a serious environmental issue (Chon et al., 2011). Therefore different methods have been developed to minimize the sludge production and to reduce the excess sludge. Aerobic and anaerobic digestion are commonly used methods in most municipal waste water treatment plants. Aerobic digestion is widely used for sludge reduction (Gronroos et al., 2005). In sludge digestion, hydrolysis is considered to be the major rate limiting step which can be overcome by sludge disintegration pretreatment methods. The biodegradability of the sludge can be improved by a variety of pretreatment methods such as thermal treatment (Jolis et al., 2004), chemo mechanical (Uma et al., 2012), electrolysis (Yuan et al., 2011), enzymatic or microbial pretreatment (Gopi Kumar et al., 2012). Among these, biological stabilization (enzymatic or microbial pretreatment) is considered to be most attractive method for reducing the major portion of the organic fraction in WAS (Kim et al., 2002). However enzymatic treatment of WAS demands high cost (Erikkson et al., 2002).Thus, culturing hydrolytic enzyme secreting microorganisms can be an efficient way to circumvent this problem. However, to enhance the sludge solubilization it is necessary to optimize the bacterial growth conditions (pH, temperature and time) before pretreatment. Conventional methods of bacterial growth conditions optimization by changing one independent variable and keeping all other variables constants are unreliable, expensive and time consuming. Response surface methodology (RSM) is a collection of statistical techniques for model

* Corresponding author. Address: Department of Civil Engineering, Regional Centre of Anna University, Tirunelveli 627007, India. Tel.: +91 9444215544.
E-mail address: rajeshces@gmail.com (J. Rajesh Banu).

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building, designing experiments and optimizing a response influenced by several independent variables. RSM is well suited to study the effects and interactions of distinct factors regarding dry cell weight of bacterial growth conditions.

Extracellular polymeric substances (EPS) are secreted by microorganisms, play an important role in bioflocculation process by interacting with the solids (Garnier et al., 2005). Hence it is essential to remove the EPS before pretreatment to reduce the organic solids. Most of the organic molecules and cations are embedded in the extracellular polymeric matrix of excess sludge which contributes to flocculation (Yu et al., 2009). Several physical and chemical methods have been reported to remove EPS associated with cells from different sources such as biofilm, sludge and cell suspension. Common physical methods include centrifugation, ultrasonication and heating. The chemical methods include removal of EPS with chemical agents such as EDTA, formaldehyde and NaOH. The chemical methods were found to be superior to physical methods. In the present study the chemical agent EDTA is used for removal of EPS. EDTA removes the cations (Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}) from the flocs structure (Wawrzynczyk et al., 2007) and disrupts the sludge matrix releasing the enzymes (adsorbed and immobilized) and other adsorbed organic nutrients into the sludge. Further pretreatment with enzyme secreting bacterial strains utilize the released substrates and enhances aerobic digestion efficiently.

The prime objective of this study was (1) to remove EPS responsible for bioflocculation with suitable cation binding agent (EDTA) and to accelerate the sludge enzyme activity at its lower concentration, (2) to investigate the effect of deflocculated sludge (EPS removed) on bacterial pretreatment and (3) to evaluate the performance of the aerobic digestion through the stabilization of sludge succeeding the enzymatic bacterial pretreatment. The kinetic equations were tested for their fitness of the hydrolysis data, in order to demonstrate the enhancement of enzyme secreting bacterial strains on sludge hydrolysis.

2. Methods

2.1. Sample collection

The Waste activated sludge (WAS) used in this study was obtained from secondary clarifier of a municipal waste water treatment plant in Trivandrum, Kerala. The sludge was concentrated by settling at 4 °C for 24 h. The initial characteristics of WAS were pH = 6.5, TCOD = 10,000 mg/L, SCOD = 100 mg/L, TSS = 7000 mg/L, VSS = 5600 mg/L.

2.2. Bacterial strains

The consortium of two bacterial strains (*Bacillus jerish 03* and *Bacillus jerish 04*) isolated from municipal waste activated sludge in the previous work were used in the present study. These strains enhance the substrate utilization through combination of enzymes protease and amylase and enhance the sludge solubilization. The mixed cultures leads to higher enzyme production (Guo and Xu 2011) which could further enhance the sludge solubilization.

2.3. Optimization of growth conditions for bacterial strains by RSM

Bacterial growth conditions were optimized using RSM. A Central composite design (CCD) made with Design – Expert software 8 was employed to investigate the simultaneous effect of three independent variables: pH, temperature and time. In this study the optimum pH, temperature and time for the growth of consortium was analysed through dry cell weight measurement of bacterial growth on nutrient broth. The total number of experimental run was 20. Based on the preliminary experiments, pH, temperature and time were varied in the range of (4–9), (20–70 °C) and (0–72 h) respectively. The target response was dry cell weight (g/L) of bacteria. To describe the response in the optimum CCD region of 3 factors for a full 2^3 factorial design with six central points, six axial points (with an axial design of ±1.63 forming an orthogonal design), and eight factorial points, twenty sets of experiments were planned .

2.4. Screening of bacterial strains for enzyme activity

The Bacterial strains were screened for enzymes such as protease and amylase through streaking on skimmed milk agar and starch agar medium, respectively. A clear halo zone appeared around the bacterial strains evidencing the secretion of the enzymes (protease and amylase). The composition of skimmed milk agar medium comprised the following: agar 20 g, pancreatic digest of casein 5 g, yeast extract 2.5 g, glucose 1 g, skimmed milk powder 10 g/100 mL, distilled water 1 L. The starch agar medium comprised the following: soluble starch 5 g, yeast extract 1 g, tryptone 2 g, $Cacl_2$ 0.003 g, $MgCl_2$ 0.1 g, KH_2 PO_4 0.36 g, Na_2HPO_4 1.3 g, agar powder 20 g, distilled water 1 L.

2.5. Cultivation of bacterial strains

The strains were cultivated in 1 L conical flask with 500 mL of nutrient broth at pH 6.5, temperature 40 °C with a shaking speed of 150 rpm for 42 h. After cultivation the cultured cells were harvested at early exponential phase (42 h) and was used as inoculum for WAS solubilization.

2.6. Optimization of bacterial dosage for sludge solubilization

100 mL of sludge were taken in five identical 250 mL conical flasks. Various dosages (1, 2, 3, 4, 5 g dry cell weight/L) of bacterial strains were inoculated into each flask. The flasks were incubated at 40 °C for 42 h. SS reduction and COD solubilization were monitored to get the optimized dosage of bacterial strains for sludge solubilization.

2.7. Optimization of dosage of EDTA for removal of EPS

100 mL of sludge was taken in seven identical conical flasks. EDTA was added to each conical flasks with its dosage ranges from (0.05 to 1 g/g SS) respectively. The conical flasks were incubated for 3 h at 4 °C (Ramdani et al., 2012). After incubation the samples were centrifuged at 10,000*g* for 15 min. The pellet was discarded. The supernatant was filtered through 0.45 μm cellulose acetate membrane to get the cell free soluble EPS. The obtained soluble EPS were biochemically characterized.

2.8. Bacterial pretreatment

100 mL of deflocculated sludge (EPS removed) was taken in 250 mL conical flask. The removal of EPS with EDTA shifts the pH of the medium to acidic condition. So that the pH of the medium was adjusted to 6.5 with 0.1 N NaOH to make it suitable for bacterial inoculation. Two gram dry cell weight g/L of bacterial strains was inoculated into it. The conical flasks were incubated at 40 °C for 42 h at 150 rpm. In the same way 100 mL of sludge was taken in another two conical flasks, one was maintained as control and other one was maintained as flocculated (without EPS removed and treated with bacteria alone) in order to study the efficiency of EPS removal in bacterial pretreatment. These two flasks were also incubated at 40 °C for 42 h.

2.9. Aerobic digestion

Aerobic digestion was performed in two identical poly vinyl chloride (PVC) reactors with a working volume of 5 L. The DO inside the reactors was maintained in the range of 2.5–3 mg/L with diffused aerators to supply pure oxygen to improve the SS degradation (Francisco et al., 2012). Among the two reactors, one was designated as 'C' (control) and other as 'E' (experimental). Control reactor was filled with 5 L of WAS sludge while the experimental reactor contained a mixture of WAS sludge plus EPS removed and bacterially pre-treated sludge in the ratio of 1:1. Hwang et al. (2007) also used similar inoculum substrate ratio while working on aerobic digestion of chemically pretreated sludge. Raw sludge serves as the substrate for microbes. Therefore 2.5 L of substrate was apt to carry out aerobic digestion efficiently. Total running time was 20 days. The samples were analysed every two days interval.

2.10. Analytical methods

Suspended solids, volatile solids, TCOD, SCOD were measured according to standard methods (American Public Health Association (APHA), 2005). The protein was quantified by Lowry's method with bovine serum albumin as the respective standard (Takahashi et al., 2009). The carbohydrate was quantified by Anthrone–sulphuric acid method with glucose as the respective standard (Tapia et al., 2009). The DNA was quantified by diphenylamine colorimetric method with *Escherichia coli* DNA as the respective standard (Boonaert et al., 2001). The total amount of removed EPS was measured by the sum of proteins and polysaccharides. All the experiments were done in triplicate.

2.10.1. Enzyme assay

The activated sludge mixed liquor was centrifuged at 10,000 rpm for 10 min and the supernatant served as the crude enzyme source. For protease activity the substrate casein (2%) was pre-incubated in water bath at 37 °C for 5 min. The assay mixture contained 0.5 mL of casein (2%) as substrate and 0.5 mL of sample (enzyme source). The reaction mixture was incubated at 37°C for 20 min. 3 mL of 5% Trichloro acetic acid was added to terminate the enzyme reaction, and was centrifuged at 5000 rpm for 5 mins and the supernatant was obtained. To 1 mL of supernatant, 2 mL of 0.5 N NaOH and 0.6 mL of 1:2 diluted Folin phenol reagent were added and incubated at room temperature for 10 min. The absorbance was measured at 660 nm. One unit of absorbance is expressed as 1 U/mL protease activity.

For amylase activity 1 mL sample (enzyme source) was mixed with 1 mL of 1% soluble starch in citrate–phosphate buffer (pH6.5).The reaction mixture was incubated in a water bath at 40 °C for 30 min. After incubation, 2 mL DNS reagent was added to terminate the reaction. Then the mixture was boiled for 5 min, cooled and mixed with 20 mL of distilled water. The absorbance was measured at 540 nm. One unit of absorbance is expressed as 1 U/mL amylase activity.

3. Results and discussion

3.1. Optimization of growth conditions for bacterial strains by RSM

The efficiency of bacterial pretreatment is linked to bacterial activity which is influenced by a variety of parameters such as pH, temperature, and time (Burgess and Pletschke, 2008). It is highly impossible to optimize the growth of inoculated organisms in the sludge as it contains numerous other organisms. Hence the optimization experiments were carried out by inoculating the desired organisms in nutrient broth and measuring its growth. Taking into consideration of strong correlation between the factors pH, temperature and time on bacterial growth, optimization in term of dry cell weight was assigned as a suitable response factor.

The analysis of the variance for the response surface quadratic model of dry cell weight measurement shows that the calculated R^2 was found to be 0.991 which indicated a high degree of correlation between predicted and observed values. The adjusted and predicted R^2 were found to be 0.983 and 0.928 which indicated that predicted R^2 was in reasonable agreement with adjusted R^2. Anova of the regression demonstrated that the model was significant. Model constructed for growth condition was found to be adequate for prediction within the range of variables applied. The values of prob >F less than 0.05 indicated that the model was significant. This implies that the effects of pH and the interactions of pH with temperature, temperature with time and pH with time had significant influence on bacterial growth.

The three dimensional response surfaces and their respective plots were shown in Fig. 1a–c. It was plotted to understand the interaction of various variables and to locate the optimal level of each variable for maximal response. The three dimensional response surface plots were generated to investigate the interactions between pH, temperature and time and to visualize their combined effects on the response of dry cell weight of bacteria as shown in Fig. 1a–c. From the graphs the combined effects of pH, temperature and time on the response of bacterial growth were observed. The growth factors such as pH, temperature and time control the growth of bacteria. Hence a strong correlation between them for effective growth is inevitable. Maximum desirability of 100% was achieved at temperature 40 °C, pH 6.5 and time of 42 h showing dry cell weight measurement of about 1.79 g/L at the central region. The RSM results indicate that the three variables studied have a significant effect on bacterial growth. There was an enhancement in dry cell weight at the central points when they were inspected with the response of pH, temperature and time as variables. The model revealed that the central point offer the actual process optimization region. The central point corresponds to pH 6.5, temperature of 40 °C and time of 42 h.

3.2. Optimization of bacterial dosage for sludge solubilization

The optimum bacterial dosage for sludge solubilization was found to be 2 g dry cell weight/L. On increasing the bacterial dosage from 1 g to 2 g dry cell weight/L, an increase in SS reduction and COD solubilization (data not shown) was observed. Further increase in the bacterial dosage did not show any significant difference in SS reduction and COD solubilisation. Insufficient supply of substrates to the bacterial inoculum at 3 g/L might have generated competition between the inoculated bacterial strains and the indigenous aerobes present in the sludge for the available substrate resulting in suppressed growth and decreased efficiency (Lee et al., 2009). The competition also resulted in the utilization of released soluble organic matter which can be evidenced by decrease in soluble organics in the medium.

3.3. Optimization of EDTA dosage for removal of EPS to enhance bacterial pretreatment

Park and Novak (2007) have reported that EPS rather than cells represents major organic fraction determining the floc structure, integrity, and strength. The disruption of Floc matrix by removing the EPS releases the entrapped or adsorbed enzymes (protease and amylase) into the sludge, enhancing the efficiency of sludge pretreatment during aerobic digestion. EDTA removes the divalent cations firmly holding the EPS with sludge, resulting in deflocculation. The total amount of EPS and DNA contents in the filtrate was

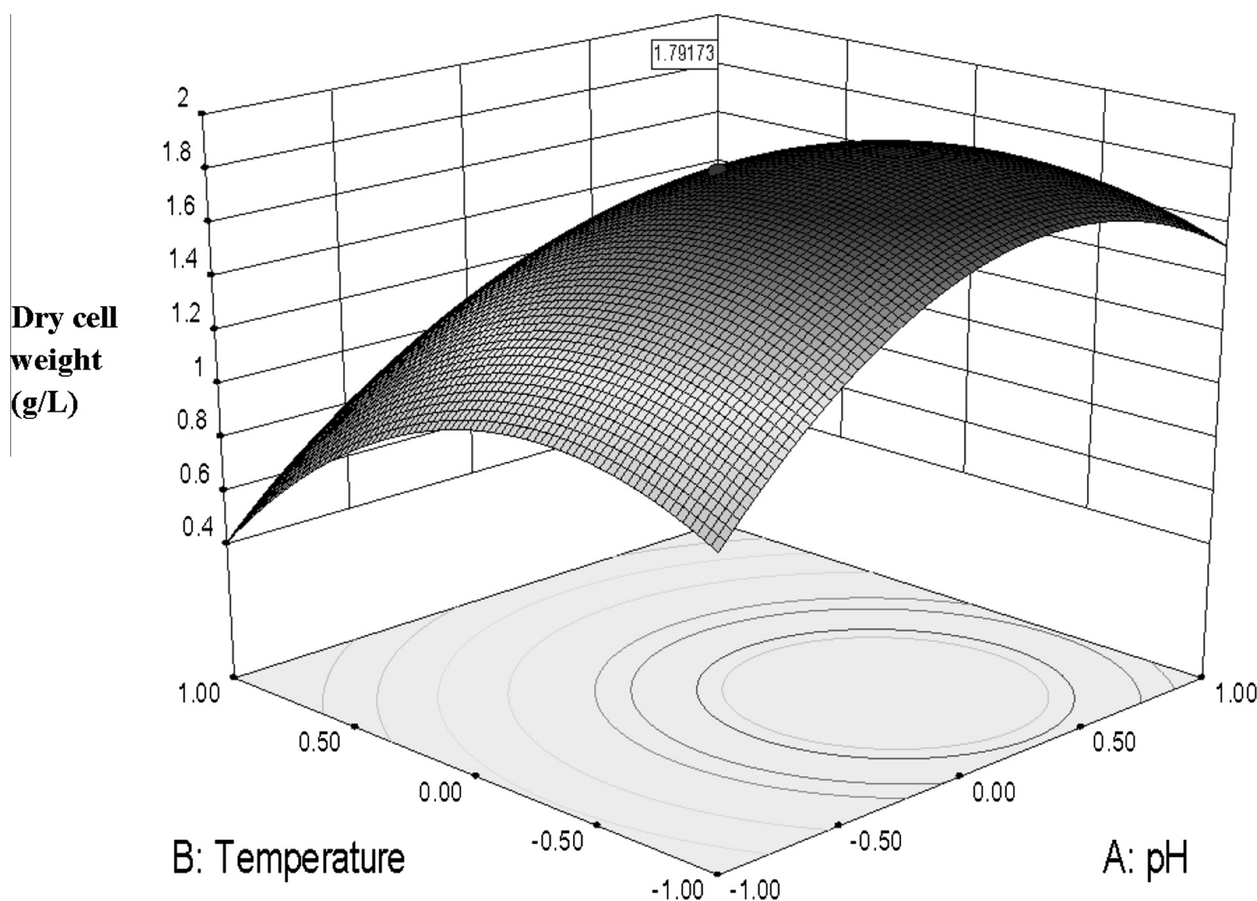

Fig. 1a. Response surface plot showing optimum bacterial growth conditions as a function of pH and temperature.

biochemically characterized and the results were depicted in Fig. 2. From the figure it was observed that there is a stepwise increase in EPS and DNA removal up to an EDTA dosage 0.2 g/g SS. At 0.2 g/g SS of EDTA dosage the concentration of EPS and DNA were found to be 40 mg/L and 8 mg/L followed by a stabilization in the removal of EPS with respect to the increase in concentration of EDTA dosage. In the previous study Merrylin et al. (2011) have reported DNA as a marker for choosing the optimum dosage of EDTA for removal of EPS. The results of the present study also showed a sharp increase in DNA when EDTA dosage increased from 0.2 to 0.3 g/g SS indicating the harsh removal condition which leads to cell breakage and release of intracellular contents. From the above findings it was concluded that 0.2 g/g SS of EDTA was found to be optimum for removal of EPS efficiently with limited cell lysis. Many researchers have studied the effects of toxic organic compounds on the microbial community of activated sludge. Microbial community and enzyme activity were two sensitive catalogues for activated sludge performance in biological treatment process. Toxic compounds might affect microbial growth (Li et al., 2013). So, the effect of EDTA on sludge enzyme activity (protease and amylase) was investigated and is depicted in Fig. 2. It was observed that there was stepwise increase in both protease and amylase activity up to 0.2 g/g SS dosage of EDTA and the corresponding enzymatic activity were found to be 0.061 and 0.035 U/mL, respectively. This might be due to disruption of floc structure which releases the enzymes adsorbed on sludge matrix. These enzymes (protease and amylase) were activated by metal ions such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+}, respectively. Some of these cations found to form complexes with the enzymes that were released into the medium (Guangui et al., 2009). Thus, the activity of these metal dependent enzymes protease and amylase increased up to 0.2 g/g SS of EDTA dosage. However, when the EDTA dose was increased above 0.2 g/g SS, the protease activity was stabilized while a decrease in the amylase activity was observed. This might be due to metal chelating capacity of EDTA (Kim et al., 2002). Further increasing the EDTA dosage could removes cations that were found to be bound with enzymes resulted in disruption and inhibition of enzyme activity. Therefore 0.2 g/g SS of EDTA was found to be optimum for sludge enzyme activity. Lower concentrations of EDTA in the range of 0.1–0.8 g have no impact on the environment (Merrylin et al., 2011). It was observed that the EDTA used for removal of EPS, degraded remarkably by inoculated enzyme secreting bacterial strains. This was affirmed through plating technique. Thus there will be no accumulation of EDTA in sludge. In addition, EDTA shifts the pH of the sludge to acidic condition. So the pH of the sludge has to be adjusted to optimized pH condition 6.5 using alkali for the subsequent bacterial pretreatment. In the present study, the pH of the sludge was found to be 5.4 when 0.2 g/g SS of EDTA was added. Further increase in EDTA dosage shifted the pH of the medium to below 4 which was practically impossible to get neutralized for subsequent pretreatment.

3.4. Bacterial pretreatment

3.4.1. Effect of bacterial pretreatment on suspended solids reduction and COD solubilization

The efficiency of both the flocculated and deflocculated sludges subjected to bacterial pretreatment was analysed on the basis of SS reduction and COD solubilization Fig. 3a depicts the effect of bacterial treatment on SS reduction. The SS reduction was significant

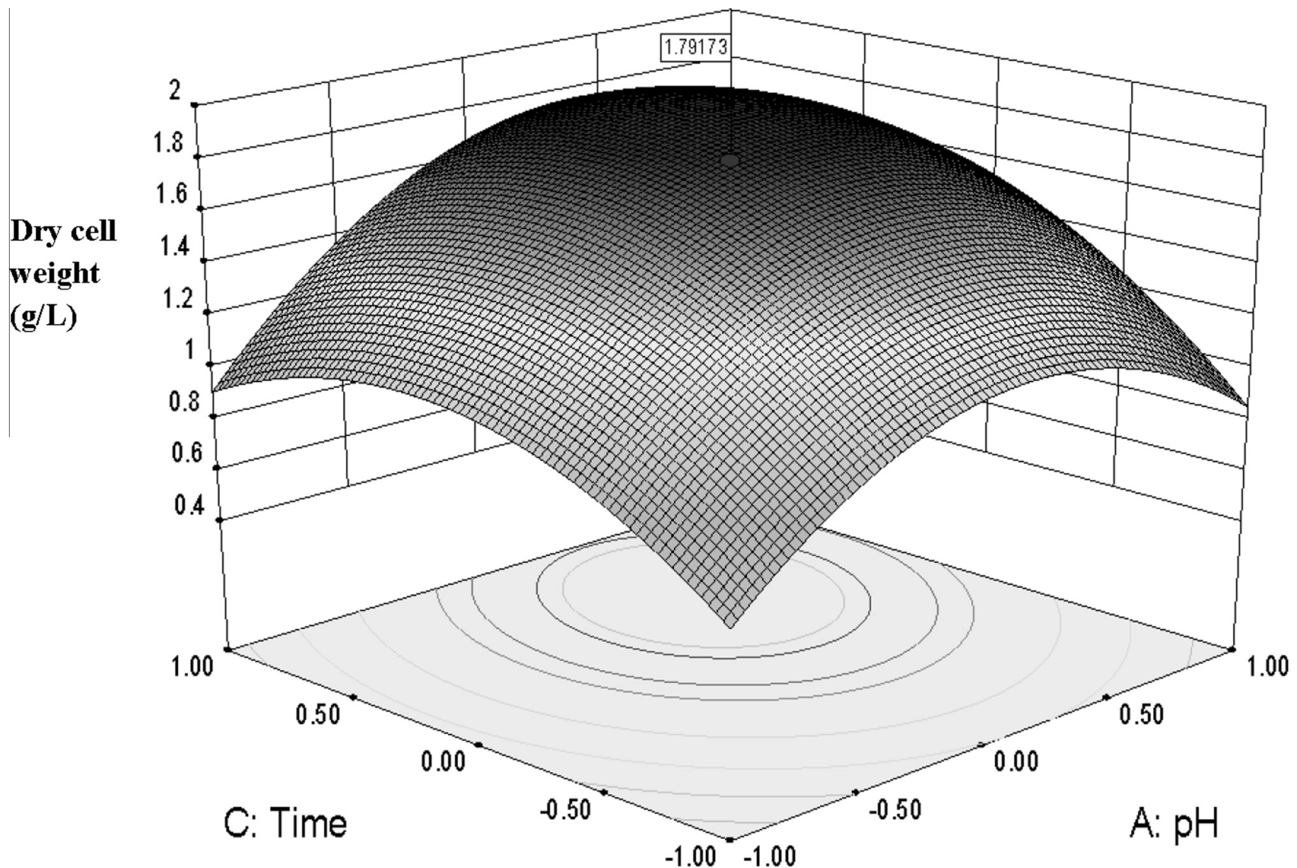

Fig. 1b. Response surface plot showing optimum bacterial growth conditions as a function of pH and time.

up to 42 h showing 7.8%, 15.7% and 24% SS reduction at control, flocculated and deflocculated sludge, respectively. The combinatorial enzyme activity of both the inoculums and those entrapped enzymes released from sludge matrix by EDTA might be the reason for high SS reduction in deflocculated sludge. After 42 h the SS reduction became stable. This may be due to saturation of microbial activity (Tang et al., 2012).

Similar to SS reduction, the COD solubilization (Fig. 3a) also increased up to 42 h showing 6%, 11% and 17.33% in control, flocculated and deflocculated sludge, respectively. Release of intracellular materials into the medium caused by enzymatic cleavage of cell walls of biomass might have increased COD solubilization. Cadoret et al. (2002) reported that the sludge solubilization depends on the diffusion of enzyme surface active sites into the sludge matrix particles. Moreover they reported that EPS can reduce the contact between the enzyme and the substrate as well as the diffusion efficiency of the substrate in the EPS matrix. Therefore the disruption of floc matrix by removing EPS and subsequent bacterial pretreatment inevitably leads to the enhancement of sludge solubilization. Hence greater solubilization in deflocculated sludge may be due to the availability of greater surface area for the action of the bacteria and the subsequent release of entrapped organic matter from the flocs. In addition, EDTA enhances the release of bound enzymes into the medium subsequently increasing cell disruption and COD solubilisation. After 42 h there was a gradual decrease in COD solubilization. This might be due to active cryptic growth of microbes i.e., microbial growth on solubilised lysates of WAS (Liu, 2003). The addition of EDTA added organic load to the sample. The theoretical organic load of EDTA was calculated and subtracted from the SCOD released to obtain the actual SCOD.

3.4.2. Effect of bacterial pretreatment on protein and carbohydrate release

As mentioned earlier, proteins and carbohydrates are the main constituents of the sludge, the WAS solubilisation efficiency of Bacterial strains (*jerish 03* and *jerish04*) was evaluated in terms of soluble protein and carbohydrate. The profile of soluble protein and carbohydrate were depicted in Fig. 3b. As expected, the concentrations of these soluble organics were much higher in deflocculated sludge than the flocculated and control. The maximal protein release were 50, 200 and 400 mg/L for control flocculated and deflocculated sludge, respectively. The carbohydrate released were 16, 60 and 80 mg/L, respectively. With the increase in treatment time of above 42 h, the soluble protein was hydrolysed into amino acids and peptides by protease (Xuesong et al., 2009) and soluble carbohydrates were hydrolysed into simple sugars due to which the soluble protein and carbohydrate concentration fell down after 42 h.

3.4.3. Effect of bacterial pretreatment on enzyme activities

The protease and amylase activities of control, flocculated and deflocculated sludges were illustrated in Fig. 3c. Throughout the pretreatment, the enzyme activities (protease and amylase) of deflocculated sludge were higher than flocculated and control sludge. Protease and amylase activity increased rapidly up to 42 h and was found to be 0.04, 0.08, and 0.18 U/mL for control, flocculated and deflocculated sludge, respectively. The increase in protease and amylase activity may be due to utilization of carbon, nitrogen sources. A greater secretion of enzymes in complex media (municipal wastewater sludge) could be due to poor accessibility of various nutrients, which would otherwise be easily assimilated. Further, these enzymes could break big complex molecules to simpler ones and release them in the medium (from sludge solid phase

Fig. 1c. Response surface plot showing optimum bacterial growth conditions as a function of temperature and time.

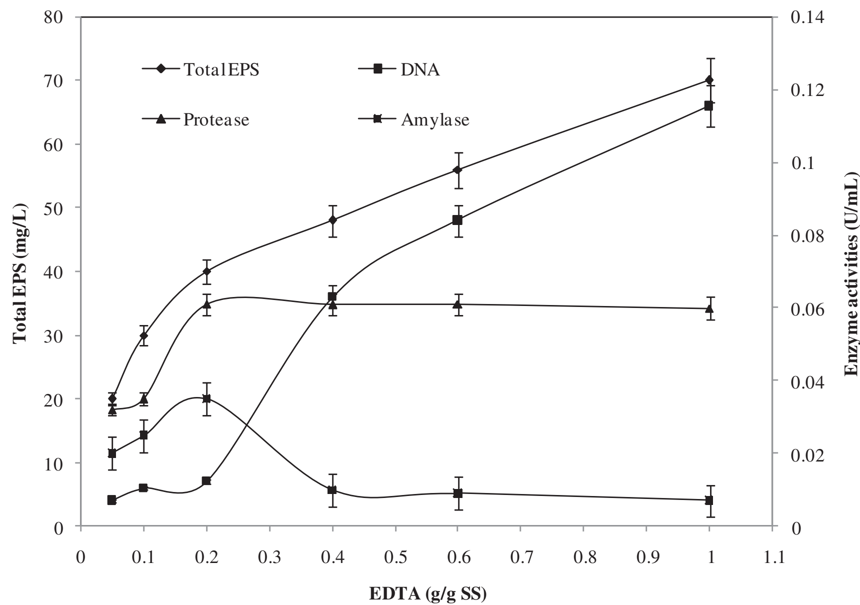

Fig. 2. Optimization of EDTA dosage for removal of extracellular polymeric substance (EPS); standard deviations are represented by error bars.

to liquid phase) enhancing accessibility. Hence, a more complex medium stimulates the bacteria to show high enzyme activity (Chenel et al., 2008). The higher protease and amylase activity in deflocculated sludge was attributed to EPS removal which released the trapped enzymes and organic molecules from the sludge matrix into the medium. These organic substances were utilized by inoculated enzyme secreting bacterial strains as their food (Lee et al., 2009) inducing the generation of more protease and amylase

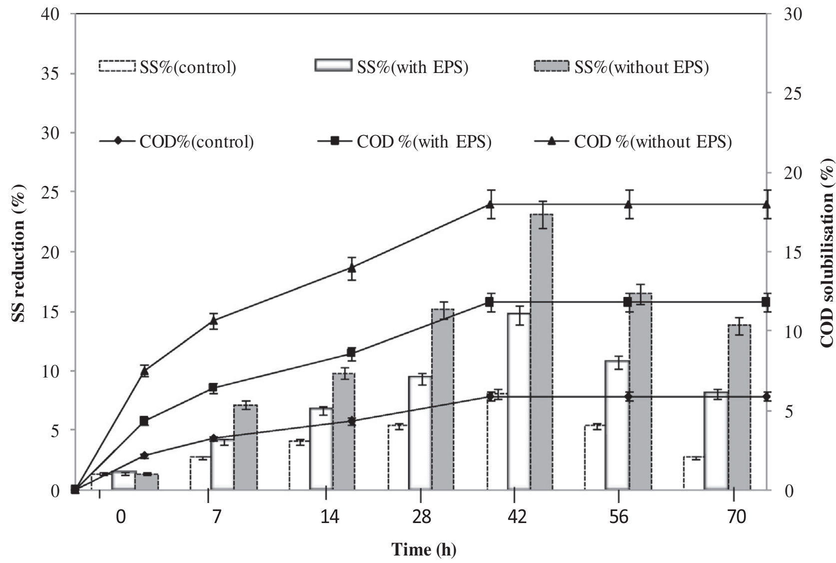

Fig. 3a. Effect of bacterial pretreatment on SS reduction and COD solubilisation; standard deviations are represented by error bars.

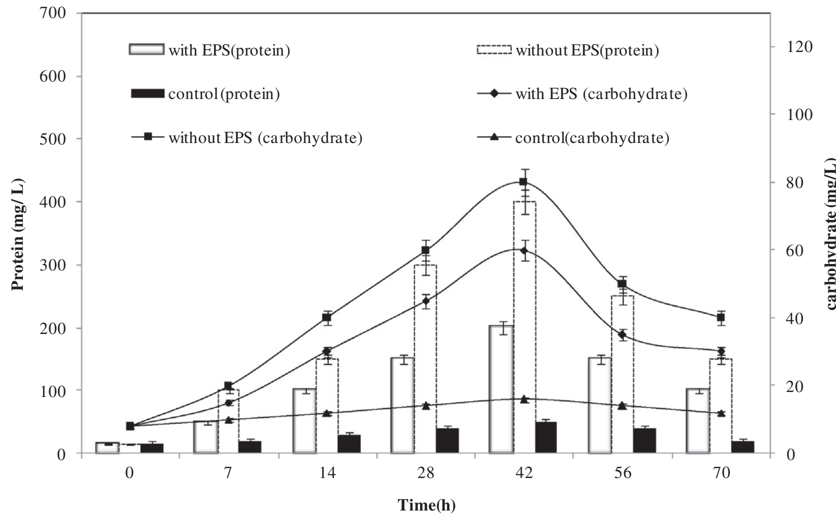

Fig. 3b. Effect of bacterial pretreatment on protein and carbohydrate release; standard deviations are represented by error bars.

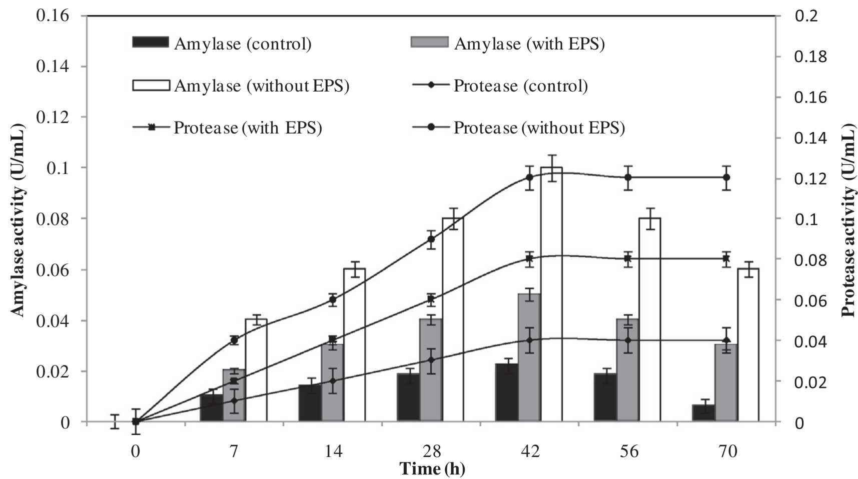

Fig. 3c. Effect of bacterial pretreatment on enzyme activities; standard deviations are represented by error bars.

Table 1
Kinetic analysis of SS and PCOD reduction enhanced by removal of EPS followed by bacterial pretreatment.

Sample	Dynamic equation		Rate constants k (h^{-1})		Coefficients R^2	
	SS reduction	PCOD reduction	SS	PCOD	SS	PCOD
Control	$Y = -0.001x - 0.010$	$Y = -0.001x - 0.011$	0.001	0.001	0.944	0.986
Flocculated (with EPS)	$Y = -0.003x - 0.021$	$Y = -0.002x - 0.010$	0.003	0.002	0.940	0.978
Deflocculated (without EPS)	$Y = -0.006x - 0.040$	$Y = -0.004x - 0.016$	0.006	0.004	0.928	0.988

SS – suspended solids, PCOD – particulate chemical oxygen demand.

enzymes as they are the excellent enzyme producers. Thus, the activity of the supernatant implies the release of the protease from inside the cell by cell lysis. Usually protease an intracellular enzyme is considered to be responsible for the endogenous respiration of the sludge and these intracellular proteases would not contribute to the sludge reduction, when released,these proteases hydrolyse the proteins in the sludge, providing the substrates for the microbial cryptic growth. However, the activity of protease and amylase become stable and decreased after 42 h due to depletion of nutrient sources.

3.5. Kinetic analysis of bacterial pretreatment

A variation in SS and particulate COD with time were observed during sludge pretreatment. The cumulative effects of these reactions were simplified to single first order kinetics. The SS reduction in control, flocculated and deflocculated sludges were found to be increased up to 42 h above which the SS reduction became stable (Fig. 3a). Similar trend was also for Particulate COD reduction (PCOD). In the present study, the sludge suspended solids and PCOD was maintained identical for all experimental condition. Other studies reported that rate of the reaction increases with increase in temperature (Luo et al., 2012). But the effect of reaction rate was not always correlated to temperature. Higher temperature would hold back the activity of neutral protease and α amylase and kill the fermentative bacteria in the sludge (Xiong et al., 2012). In the present study bacterial pretreatment reaction was carried out at temperature 40 °C which is considered to be mesophilic and it won't affect the sludge enzyme activity and other microbes present in the sludge and the inoculated strains whose optimum temperature was found to be 40 °C. PCOD can be calculated by subtracting SCOD from TCOD. Based on the above descriptions, the effect of

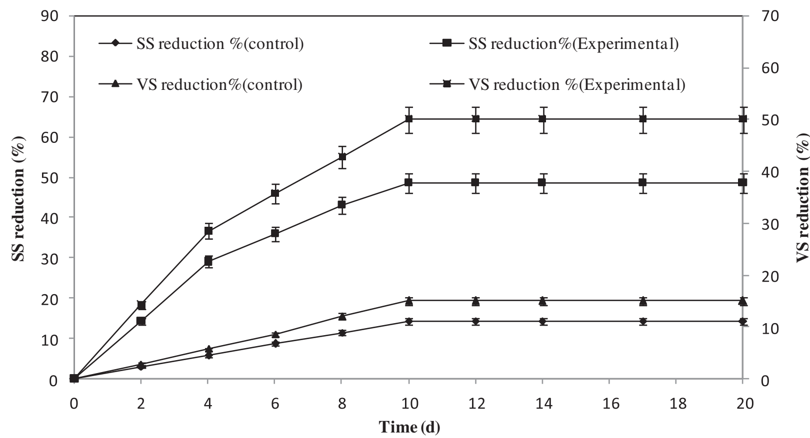

Fig. 4a. Effect of aerobic digestion on solids reduction; standard deviations are represented by error bars.

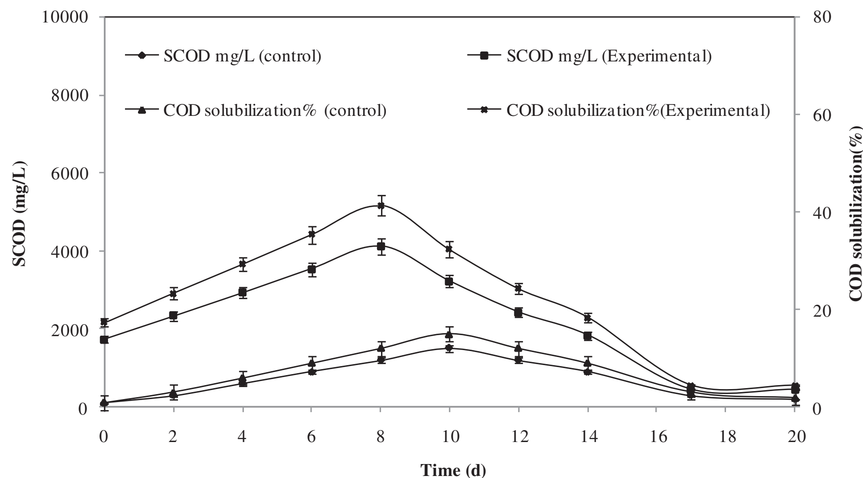

Fig. 4b. Effect of aerobic digestion on COD solubilization; standard deviations are represented by error bars.

bacterial pretreatment on SS reduction and PCOD reduction within initial 42 h at temperature 40 °C and at bacterial dosage 2 g dry cell weight/L could be expressed by the first order reaction.

By plotting the graph, ln value of SS reduction and PCOD reduction versus time, slope and intercept could be obtained The kinetic parameters were illustrated in Table 1 As shown in Table 1 the *k* value of deflocculated and bacterially pretreated sludge was greater than the flocculated sludge and control sludge. The rate constants of control, flocculated and deflocculated sludges were 0.001, 0.003 and 0.006 respectively. The goodness of fit values for control flocculated and deflocculated sludges were within the range of 0.92–0.99 recommending good fit. The goodness of fit was in complete agreement with Luo et al. (2012).

Similarly the kinetic parameters for PCOD reductions were also estimated and are illustrated in Table 1. As shown in Table 1, the *k* value of deflocculated and bacterially pretreated sludge was greater than the flocculated sludge and control. The rate constants of control, flocculated and deflocculated sludges were 0.001, 0.002 and 0.004 h^{-1} respectively. The goodness of fit values for control flocculated and deflocculated sludges were within the range of 0.92–0.99 recommending good fit.

3.6. Aerobic digestion

3.6.1. Effect of aerobic sludge digestion on solids reduction

The effect of sludge treatment on solids reduction during aerobic digestion was evaluated (Fig. 4a). A significant reduction in SS and VS was observed up to 10 days in experimental reactor showing 48.5% and 50%, respectively. The reduction was much higher than the control reactor which showed SS and VS reduction of 14.2% and 15%, respectively. A 34.3% increase in SS reduction was observed in the present study. The findings of the present study are comparable to Yu et al. (2008) they observed 35% increases in SS reduction during aerobic digestion of ultrasonically pretreated sludge. However, the bacterial pretreatment is considered superior over ultrasonic pretreatment as they are cost effective. An SRT of 10 days is appropriate for aerobic digestion. The SS reduction became stable after 10 days indicating the depletion of nutrients, carbon sources and extracellular enzymes.

3.6.2. Effect of aerobic sludge digestion on COD solubilization

The variation in COD solubilisation is depicted in Fig. 4b. A sharp increase in SCOD was observed in both the reactors up to 10 days after which it started decreasing. The experimental reactor showed higher COD solubilisation of 47.3% than the control reactor (15%). Initial increase in solubilisation was due to susceptibility of the pretreated sludge to enzymatic action and release of intracellular materials into the aqueous medium (Kim et al., 2002; Banu et al., 2009) and later the decrease in solubilisation was plausibly due to the assimilation of soluble organics by the bacteria. Though the SS reduction was achieved in 10 days, it cannot be considered optimum for aerobic digestion as a major portion of SCOD remains in sludge after 10 days. As the SCOD reaches 450 mg/L on 17th day, SRT of 17 days can be decided optimum for aerobic digestion.

4. Conclusion

The bacterial consortium (*Bacillus jerish 03*Accession number KC597266 &*Bacillus jerish 04*Accession numberKC597267) secreted extracellular enzymes (protease and amylase) which could solubilise the sludge .EDTA dose of 0.2 g/g SS potentially removed the EPS and enhanced the sludge pretreatment. The bacterial pretreatment of deflocculated sludge improved the sludge reduction in aerobic digestion. SS reduction was significant in aerobic digestion showing an increase of about 34.3% in bacterially pretreated sludge. The present technology is cost effective and environmental friendly and can be applicable for field study. Similar study to evaluate the application of the consortium in anaerobic digestion of WAS is essential.

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